



Increased serum CK-MB/ CK ratio and its relation to serum uric acid with rotavirus gastroenteritis

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ABSTRACT

Background: Rotavirus gastroenteritis (RVG) is higher serum uric acid (s-UA) than other viral gastroenteritis while its pathogenesis remains unclear. Mitochondrial creatine kinase (mtCK), a creatine kinase (CK) isozyme, is reported to be higher in patients with RVG, possibly due to breakdown of intestinal cells. To test the hypothesis that increased serum UA levels with RVG are due to damage to the intestinal cells, which is characterized by concomitant elevation of serum mtCK.

Methods: Overall 41 patients had RVG, 29 had norovirus gastroenteritis, 10 had adenovirus gastroenteritis, and 20 had bacterial gastroenteritis. As it is known that mtCK falls within the fraction of creatine kinase-MB (CK-MB) assayed by immunoinhibition method, the CK-MB/CK ratio was compared among the groups to elucidate the levels of mtCK. Furthermore, the relationship between s-UA and the CK-MB/CK ratio was also examined.

Results: The RVG group had the highest s-UA level (median 6.7 mg/dL); and levels were significantly higher than healthy subjects (HS), pneumonia, and pharyngitis patients ($P = 0.000$). The RVG group had a significantly higher CK-MB/CK ratio than other gastroenteritis groups, HS, and disease control groups ($P = 0.000$). There was a positive correlation between s-UA and CK-MB/CK ratio in patients with gastroenteritis ($R = 0.45$, $P = 0.000$).

Conclusion: Increased serum CK-MB/CK ratio in patients with RVG indicates the increased mtCK due to tissue breakdown, particularly that of small intestines. Considering the strong relationship between s-UA and CK-MB/CK ratio, it is speculated that the increase in s-UA levels is due to intestinal mucosal injury.

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1. Introduction

Rotavirus (RV) is one of main pathogens associated with gastroenteritis in childhood.

RV gastroenteritis (RVG) is associated with higher serum uric acid (s-UA) levels than other forms of

viral gastroenteritis (Kovacs *et al.*, 1987; Palumbo *et al.*, 2009). This increase in s-UA is transient in most cases and returns to normal as symptoms subside. However, as some patients can develop obstructive uropathy (Fujita *et al.*, 2009; Ashida *et al.*, 2012), the evaluation of this parameter is important.

The following mechanisms through which RV causes hyperuricemia have been proposed: [1] increased production by tissue breakdown, particularly intestinal mucosa cell damage (Kovacs *et al.*, 1987; Kaneko *et al.*, 2010; Morita and Fujieda, 2011); [2] decreased excretion due to severe dehydration (Adler *et al.*, 1982; Kovacs *et al.*, 1987; Palumbo *et al.*, 2009); and [3] increased production by tissue breakdown of direct renal tubular damage (Kovacs *et al.*, 1987; Kaneko *et al.*, 2010; Morita and Fujieda, 2011). However, the exact mechanisms for hyperuricemia caused by RVG are yet to be elucidated.

Creatinine kinase (CK) is a dimer comprised of a B subunit and M subunit and includes three major isozymes: CK-BB, CK-MB, and CK-MM. In addition, mitochondrial CK (mtCK) has been identified which increases in infants with RVG. The proposed mechanism for this involves damage to the intestinal mucosa (Hoshino *et al.*, 2001).

This study aims to determine whether increased s-UA indicates intestinal mucosa impairment in patients with RVG by measuring mtCK levels.

2. Materials and methods

2.1 Subjects

Two-hundred sixty-seven children aged 15 years or younger diagnosed with acute gastroenteritis who visited Komatsu Hospital between January 2009 and June 2014 were enrolled in this study. Pathogen was confirmed by stool culture for bacterial gastroenteritis (BG) and faecal viral antigen test for viral gastroenteritis: RV, adenovirus (AV), and norovirus (NV) antigens were tested for using immunochromatography (RapidTesta Rota-Adeno,

Sekisui Medical Co. Ltd., Tokyo, Japan; Quick Navi TM-Noro 2, Denka Seiken Co. Ltd., Tokyo, Japan). The subjects consisted of 41 patients with RVG (median age: 2.3 years [interquartile range: 1.4-6.9 years]), 29 patients with norovirus gastroenteritis (NVG) (2.9 years [1.5-4.6 years]), 10 patients with adenovirus gastroenteritis (AVG) (3.9 years [2.2-5.0 years]), and 20 patients with BG (3.9 years [3.0-4.7 years]). Patients with mixed infections were excluded from this study.

The control subjects were 55 healthy subjects (HS) (2.8 years [1.3-5.8 years]) and disease control subjects were 59 patients with pneumonia (2.1 years [1.8-5.9 years]), 40 patients with pharyngitis (2.8 years [1.8-5.9 years]), and 13 patients with Kawasaki disease (KD) (2.5 years [1.0-2.7 years]).

The pneumonia group consisted of patients who were diagnosed with pneumonia or bronchitis by chest radiograph (28 patients were positive for respiratory syncytial virus and 5 patients were positive for human metapneumovirus). The pharyngitis group was comprised of patients with fever and redness of the pharynx or tonsils (4 patients were positive for group A β -hemolytic streptococcus and 6 patients were pharyngeal adenovirus antigen-positive). The KD group was composed of patients who met the guidelines for the diagnosis of KD, and did not include those with incomplete KD.

This study was approved by the ethical review board (approval number: 022013)

2.2 Laboratory evaluation

Blood samples were obtained from the subjects before fluid infusion or drug administration. Serum levels of UA (s-UA: Uricase-N-(3-sulfopropyl)-3-methoxy-5-methylaniline (HMMPS) method), CK (Oliver-Rosalki method), and CK-MB (immunoinhibition method) were measured in the same specimen. As it is known that mtCK falls within the fraction of CK-MB (Lee *et al.*, 1994; Hoshino *et al.*, 2009), we used the serum CK-MB/CK ratio to assess the serum level of mtCK level. The CK-MB/CK ratio was compared among the groups. The correlation between s-UA and the serum CK-MB/CK ratio was examined.

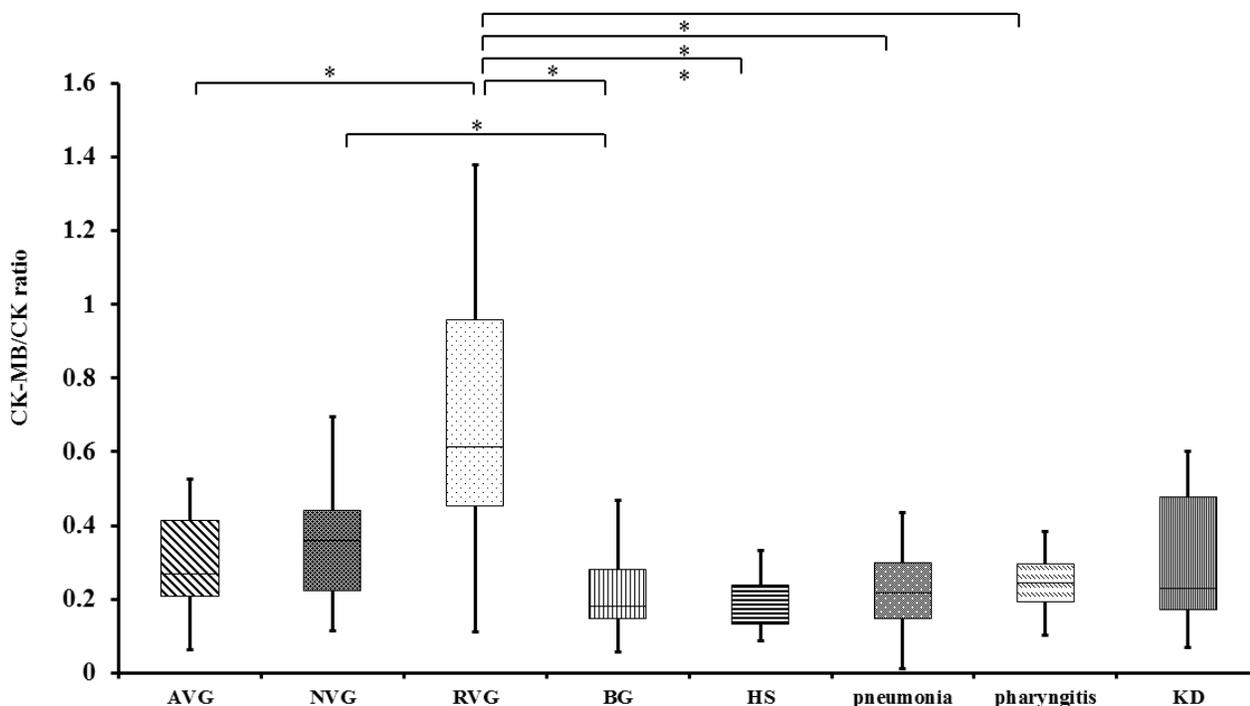


Figure 1. Comparison of the CK-MB/CK ratio in patients with gastroenteritis.

The rotavirus gastroenteritis group had a significantly higher CK-MB/CK ratio (median: 0.61; interquartile range: 0.45-0.96) ($p = 0.000$) compared to the AVG, BG, HS, and disease control. The NVG had a significantly higher ratio (median: 0.36; interquartile range: 0.22-0.44) than the HS (median: 0.17; interquartile range: 0.13-0.23). The central horizontal line represents the median value, and the bottom and top edges represent the 25th and 75th percentiles, respectively. The central vertical lines extend to the 90th and 10th percentiles. * $P < 0.01$

AVG, adenovirus gastroenteritis; NVG, norovirus gastroenteritis; RVG, rotavirus gastroenteritis; BG, bacterial gastroenteritis; HS, healthy subjects; KD, Kawasaki disease

2.3 Statistical Analysis

Data were analysed using the Kruskal-Wallis test and Spearman's rank correlation coefficient and a P-value of less than 0.05 was considered statistically significant.

3. Results

3.1 Clinical characteristics of patients

There were no significant differences in age ($p = 0.113$) or sex ($p = 0.562$) among the groups.

3.2 Summary of the routine laboratory data

Table 1 shows the summary of the data for each group: 1) a significantly higher level of white blood cells (WBCs)

was observed in the NVG, BG, pharyngitis, and KD groups compared to the HS group. Results from the NVG group were significantly higher compared to the RVG group ($p = 0.001$); 2) c-reactive protein, was significantly higher in the AVG, NVG, BG, pneumonia, pharyngitis, and KD groups compared to the HS group ($p = 0.000$); 3) the NVG group had a significantly higher blood urea nitrogen (BUN)/creatinine (Cr) ratio compared with the HS, pneumonia, and pharyngitis groups. Results for the RVG group were significantly higher than the pneumonia and pharyngitis groups ($p = 0.001$). There was no significant difference between the NVG and RVG groups; 4) in children with acute gastroenteritis, s-UA levels were highest in the RVG group, followed by the NVG group, AVG group, and BG group. In particular, the RVG and NVG groups had significantly higher levels than the HS,

Table1. Clinical characteristics of patients in each groups

	GE				Non-GE				P value
	AVG	NVG	RVG	BG	HS	pneumonia	pharyngitis	KD	
number of cases	10	29	41	20	55	59	40	13	
male gender	6	13	24	13	28	35	18	9	p=0.562
age (years)	3.87 (2.19-5.04)	2.85 (1.54-4.64)	2.28 (1.35-6.86)	3.92 (3.07-4.72)	2.76 (1.28-5.83)	2.05 (1.29-4.04)	2.75 (1.78-5.92)	2.45 (1.03-2.65)	p=0.113
WBC (x 10 ² /μl)	112 (78.8-5.133.8)	119 (88-174)	90 (67-106)	116 (85.8-160.3)	80 (71.3-5.115.8)	86 (63.5-134)	115 (83.5-145.5)	129 (95-160)	p=0.001
CRP (mg/dl)	1.0 (0.72-2.18)	0.84 (0.36-1.75)	0.2 (0.09-0.81)	0.97 (0.14-1.60)	0.04 (0.03-1.17)	1.20 (0.46-3.00)	2.27 (0.51-4.69)	3.56 (2.63-8.82)	p=0.000
BUN (mg/dL)	9.4 (5.6-11.7)	14.8 (10.8-18.5)	13.5 (9.5-19.2)	13.8 (9.0-18.7)	10.7 (9.0-14.1)	9.1 (7.8-12.1)	8.5 (6.8-11.0)	8.7 (7.8-12.1)	p=0.000
s-Cr (mg/dL)	0.27 (0.23-0.32)	0.25 (0.21-0.28)	0.27 (0.23-0.34)	0.29 (0.26-0.31)	0.27 (0.23-0.33)	0.26 (0.23-0.33)	0.28 (0.24-0.34)	0.27 (0.24-0.36)	p=0.223
BUN/s-Cr ratio	36.6 (21.1-41.1)	60.4 (39.6-82.5)	47.4 (35.6-65.6)	48.9 (28.0-65.5)	38.8 (28.5-54.1)	32.3 (25.2-44.2)	29.8 (23.4-38.1)	33.9 (31.7-45.4)	p=0.000
s-UA (mg/dl)	5.4 (3.3-5.8)	6.2 (5.0-7.3)	6.7 (5.4-7.7)	5.0 (3.9-5.5)	3.6 (3.3-4.5)	4.6 (3.6-5.7)	4.4 (3.8-4.9)	5.0 (3.8-5.1)	p=0.000
CK (IU/L)	83 (63-92)	82 (72-109)	106 (85-129)	95 (70-122)	136 (113-175)	92 (72-119)	77 (63-92)	71 (43-89)	p=0.000
CK-MB (ng/ml)	19 (14-28)	29 (22-42)	67 (49-95)	18 (16-31)	26 (20-31)	20 (16-25)	18 (15-21)	14 (13-20)	p=0.084
CK-MB/CK ratio	0.27 (0.21-0.41)	0.36 (0.22-0.44)	0.61 (0.45-0.96)	0.18 (0.15-0.28)	0.17 (0.13-0.24)	0.22 (0.15-0.30)	0.24 (0.19-0.30)	0.23 (0.17-0.48)	p=0.000

GE: gastroenteritis group Non-GE: non gastroenteritis group

WBC, white blood cell count; CRP, C-reactive protein; BUN, blood urea nitrogen; s-Cr, serum creatinine;

s-UA, serum uric acid; CK, creatine kinase; CK-MB, creatine kinase-MB

Data expressed as median(interquartile range). For the statistical analysis, the Kruskal-Wallis test was used.

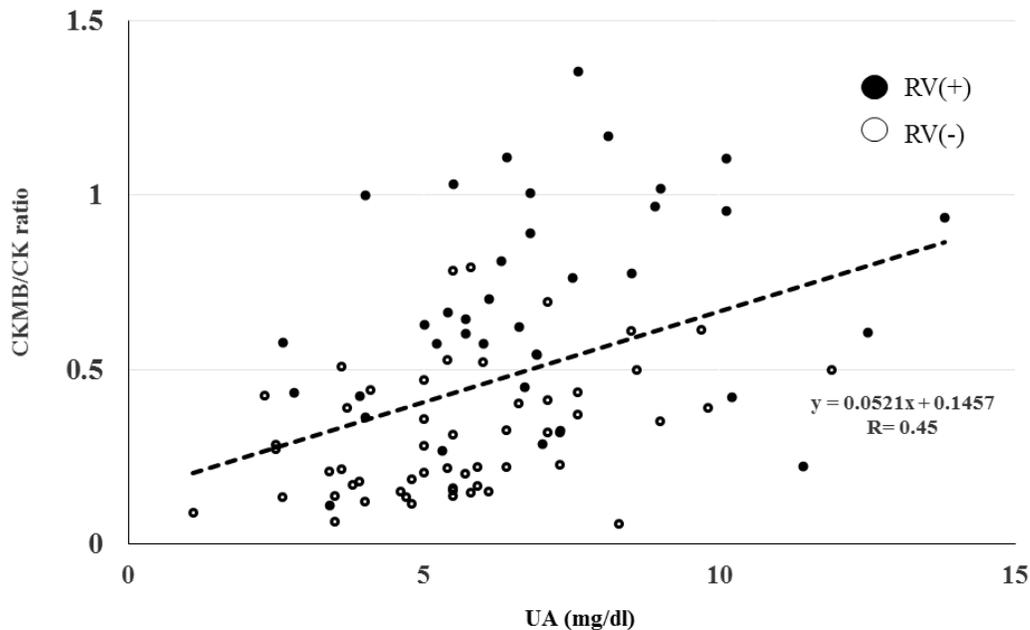


Figure 2. The relationship between CK-MB/CK ratio and UA levels in patients with gastroenteritis. Closed circle: RV positive gastroenteritis. Open circle: RV negative gastroenteritis. RV, rotavirus

pneumonia, and pharyngitis groups ($p = 0.000$).

No children developed oliguria/anuria, had no severe sequelae or died during the course of this study.

3.3 Comparison of CK-MB/CK ratio among the groups

As shown in Fig.1, the RVG group disclosed a significantly higher CK-MB/CK ratio (median: 0.61 [interquartile range: 0.45-0.96]) compared with those of AVG, BG, HS, pneumonia, and pharyngitis groups (0.27 [0.21-0.41], 0.18 [0.15-0.28], 0.17 [0.13-0.24], 0.22 [0.15-0.30], 0.24 [0.19-0.30], respectively; $p = 0.000$); the NVG group (0.36 [0.22-0.44]) showed a significantly higher ratio than the HS group (median: 0.17 [0.13-0.24]).

3.4 Relationship between CK-MB/CK ratio and s-UA levels in patients with gastroenteritis

There was a significant positive correlation between s-UA level and the CK-MB/CK ratio in patients with

gastroenteritis ($R = 0.45$, $P = 0.000$) (Fig.2).

4. Discussion

As with previous reports, our results showed higher s-UA levels in the RVG group, followed by the NVG group, compared to the HS, pneumonia, and pharyngitis groups. Two major mechanisms have been proposed for the increase in s-UA level in RVG, i.e., increased UA production, such as tissue breakdown and/or decreased UA excretion into the urine, such as dehydration (Wilcox, 1996). In our study, s-UA level was higher in the RVG group than that in the NVG group in which the severity of dehydration assessed by the BUN/s-Cr ratio was more pronounced. Similarly, Kaneko, et al. reported that hyperuricemia in RVG patients was not caused by dehydration in a comparison between patients with RVG and other types of gastroenteritis (Kaneko *et al.*, 2010). Meanwhile, Fujita et al. (Fujita *et al.*, 2009) reported that the urinary UA/Cr ratio was not decreased in patients with RVG. Taken together, it is unlikely that

hyperuricemia in RVG is associated with dehydration.

In this study, the RVG group had a significantly higher CK-MB/CK ratio compared to patients with other types of gastroenteritis and s-UA levels were also higher in this group. In addition, there was a positive correlation between s-UA levels and the CK-MB/CK ratio among the gastroenteritis groups. CK has three isozymes: CK-BB, CK-MB, and CK-MM. In addition, it has been reported that mitochondria-derived mtCK which binds to the mitochondrial inner membrane exists (James and Harrison, 1979). MtCK activity in blood can be a clinically significant tumor marker (Kanemitsu *et al.*, 1984; Rogalsky *et al.*, 1985; Suzuki *et al.*, 1986), and also be an indicator of neonatal asphyxia (Kuint *et al.*, 1993). Furthermore, Hoshino *et al.* firstly reported that mtCK levels are increased in patients with RVG (Hoshino *et al.*, 2001). They speculated that mtCK, an octamer located at the exterior of the mitochondrial inner membrane in the small intestinal mucosa, enters into the bloodstream due to cytolysis associated with RV infection (Hoshino *et al.*, 2001).

Based on these findings, we focused on serum levels of mtCK in order to clarify the causal relationship between tissue breakdown and hyperuricemia in RVG. In the present study, we used the serum CK-MB/CK ratio to assess the serum level of mtCK level because the CK-MB value, as measured by the immunoinhibition method, was substituted by the mtCK level. As a result, CK-MB/CK ratio was the highest in the RVG group among the groups suggesting the intestinal mucosal damage is most severe. In agreement with this finding, Morita *et al.* (Morita and Fujieda, 2011) suggested that loss of bicarbonic acid from small intestine epithelial cells was greater and tissue injury was more severe in patients with RVG compared to NVG. From these findings, we postulate that increased s-UA frequently observed in children with RVG is caused by increased UA production due to breakdown of intestinal cells.

There are several limitations in our study. First, actual mtCK level was not measured and therefore serum levels of CK-BB could affect the results. However, it is reported that an approximately 80% of specimens with a high CK-MB/CK ratio (> 0.25) measured by the immunoinhibition method revealed high serum levels of mtCK (Hoshino *et al.*, 2009). Furthermore, Lee *et al.* demonstrated that

mtCK was a major contributor to a total CK level under 100 IU/L in 72% of patients (Lee *et al.*, 1994). We also confirmed that all samples with elevated levels of CK-MB/CK ratio (> 0.27 , $n=21$) were within the normal range of CK-MB assessed by electro-chemiluminescence immunoassay method, which did not include mtCK (data not shown). Second, we did not directly assess the intestinal damage in this study. Considering mitochondria are present ubiquitously in any tissue, it cannot be concluded that an increase in mtCK is caused by release from the intestinal tract. However, no significant increase in the CK-MB/CK ratio in the disease control groups indicates that mtCK was released from the intestinal tract in RVG.

In conclusion, our study suggests that increased UA in patients with RVG is caused by tissue injury of the intestinal mucosa based on our measurements of the CK-MB/CK ratio in children with gastroenteritis.

Acknowledgement

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Conflict of interest

We declare that we have no conflict of interest.

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